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Investigating the heterogeneity of alkylating agents' efficacy and toxicity between genders: a systematic review and meta-analysis of randomized trials comparing cyclophosphamide and ifosfamide (MAIAGE study)

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Abbreviations

RCT	Randomized controlled trial
EFS	Event-free survival
PFS	Progression-free survival
OS	Overall survival
HR	Hazard ratio
OR	Odds ratio
95%CI	95%-confidence interval
VAC	Vincristine dactinomycin cyclophosphamide
VAI	Vincristine dactinomycin ifosfamide

ABSTRACT

Background: A marginal interaction between sex and the type of alkylating agent was observed for event-free survival in the Euro-EWING99-R1 randomized controlled trial (RCT) comparing cyclophosphamide and ifosfamide in Ewing sarcoma. To further evaluate this interaction, we performed an individual patient data meta-analysis of RCTs assessing cyclophosphamide *vs*. ifosfamide in any type of cancer. **Methods**: A literature search produced two more eligible RCTs (EICESS92 and IRS-IV). The endpoints were progression-free survival (PFS, main endpoint) and overall survival (OS). The hazard ratios (HR) of the treatment-by-sex interaction and their 95%-confidence interval (95%CI) were assessed using stratified multivariable Cox models. Heterogeneity of the interaction across age categories and trials was explored. We also assessed this interaction for severe acute toxicity using logistic models. **Results**: The meta-analysis comprised 1528 pediatric and young adult sarcoma patients from three RCTs: Euro-EWING99-R1 (n=856), EICESS92 (n=155) and IRS-IV (n=517). There were 224 PFS events in Euro- EWING99-R1 and 200 in the validation set (EICESS92+IRS-IV); and 171 and 154 deaths in each dataset respectively. The estimated treatment-by-sex interaction for PFS in Euro-EWING99-R1 (HR=1.73, 95%CI=1.00-3.00) was not replicated in the validation set (HR=0.97, 95%CI=0.55-1.72), without heterogeneity across trials (p=0.62). In the pooled analysis, the treatment-by-sex interaction was not significant (HR=1.31, 95%CI=0.89-1.95, p=0.17), without heterogeneity across age categories (p=0.88) and trials (p=0.36). Similar results were observed for OS. No significant treatment-by-sex interaction was observed for leucopenia/neutropenia (p=0.45), infection (p=0.64) or renal toxicity (p=0.20). **Conclusion**: Our meta-analysis did not confirm the hypothesis of a treatment-by-sex interaction on efficacy or toxicity outcomes.

INTRODUCTION

The Euro-E.W.I.N.G.99-R1 randomized trial (EE99-R1, NCT00020566)[1] compared the efficacy of cyclophosphamide and ifosfamide combined with vincristine and dactinomycin (VAC vs. VAI) as maintenance treatment in localized standard-risk Ewing sarcoma. We observed that sex marginally modified the treatment effect on event-free survival (EFS, interaction test, p=0.083): in males, VAC was associated with poorer EFS than VAI with a hazard ratio (HR) (VAC/VAI) =1.34 (95%CI, 0.96-1.86), whereas VAC was slightly better than VAI in females with a HR=0.83 (95%CI, 0.54-1.28).[2]

Epidemiological studies have reported a higher incidence and mortality among men than women.[3,4] Registry-based survival analyses adjusted for age and disease stage have also shown that survival tends to be worse in males in various cancers.[4,5] Moreover, numerous clinical trials of cancer patients report a worse prognosis in males in most studies.[6–10] There are also sex differences in chemotherapy-related toxicity, especially with alkylating-based chemotherapy, with higher toxicity rates in females, especially hematological toxicity.[2,10–14] Some of these findings regarding efficacy and toxicity can be explained by pharmacokinetic differences in drug metabolism (e.g. different expression of liver metabolizing enzymes according to sex), leading some authors to propose sex-based dose adaptations.[15–18]

However, no interaction between the type of alkylating agent (cyclophosphamide or ifosfamide) and sex on efficacy and acute toxicity outcomes was reported before the EE99-R1 trial. In an attempt to confirm the EE99-R1 observation, we conducted a Meta-Analysis on Interaction between Alkylating agents and GEnder (MAIAGE) of randomized controlled trials (RCT) comparing cyclophosphamide versus ifosfamide, to confirm whether or not the effect of these two treatments differs between males and females.

MATERIALS and METHODS

Trial selection

To identify an independent validation set for the EE99-R1 data, we undertook a bibliographic search of clinical trials randomizing cyclophosphamide *vs*. ifosfamide (possibly in addition to other drugs but these drugs had to be identical in both arms) in both sex, without restriction on patient age and type of cancer. We searched PubMed and The Cochrane Library for articles published between 1980 and 2013 (any language), and the National Institute of Health clinical trials register (https://clinicaltrials.gov/). In addition, all participating trialists were asked to review and supplement a provisional list of trials. Trial selection was accomplished by two authors (BF, GLT) and all relevant articles were reviewed by a third (MCLD).

Cyclophosphamide and ifosfamide could have been administered either as a single drug or combined with other drugs, but in the latter case, the only difference between the two arms had to be cyclophosphamide and ifosfamide. Differences in the dosage and infusion duration of cyclophosphamide and ifosfamide were allowed across studies. RCTs comparing only one course of cyclophosphamide or ifosfamide were not eligible. Moreover RCTs for which individual patient data concerning survival and toxicity were not available, were excluded.

Data extraction and trial quality assessment

Individual patient data were collected for each trial: sex, date of birth, allocated treatment, date of randomization, date of first event, type of first event (progression, relapse, secondary malignancy, death), date of last follow-up or death, survival status and cause of death (if applicable). We also collected acute toxicity data for leucopenia/neutropenia, thrombocytopenia, infection, mucositis and diarrhea, renal, liver, cardiac, skin, central and peripheral neurologic toxicities during the randomized period with the grade according to the NCI-CTCAE (Common Terminology Criteria for Adverse Events) grading system. Individual anonymous data were centrally collected (BF, MCLD) and checked using a standard

procedure (See Supplemental Methods S1). We noted missing data, data validity, randomization integrity and follow-up of patients between the two arms.[19]

Statistical analysis

The primary endpoint was progression-free survival (PFS), defined as the time from randomization to progression, recurrence or death from any cause, whichever occurred first. The secondary endpoint was overall survival (OS), defined as the time from randomization to death from any cause. Patients who had no events were censored at the date of the last follow-up. Analyses were performed on an intention-to-treat basis.

The validation set was analyzed using a multivariable Cox model, stratified by trial and sex, and including treatment (cyclophosphamide *vs*. ifosfamide) and age as main fixed effects. Age was divided into 3 categories (< 12, [12-18] and > 18 years) with selected cut-offs close to those defining the different pubertal status for males and females. The hazard ratio (HR) of the treatment effect by sex was measured by an interaction term ("one-stage" model).[20] Sensitivity analyses were also performed (see Supplemental Methods S2).

The heterogeneity test was assessed by Cochran's Q-statistics and I^2.[21,22] In addition, we performed an exploratory analysis on all RCTs, i.e. EE99-R1 and the validation set. Stratified PFS curves were used to calculate the absolute difference at 5 years.[23] All statistical analyses performed for the validation set were also repeated on the pooled dataset. To explore heterogeneity of the treatment-by-sex interaction term across all trials and age categories, a 3-order interaction term was included, with the relative 2-order interactions terms.

For each type of acute toxicity, the maximum grade was computed for each patient and dichotomized as follows: hematologic toxicity (<, ≥grade-4), mucositis (<, ≥grade-3), diarrhea (<, ≥grade-3) and infection, renal, liver, cardiac, skin, central and peripheral neurologic toxicities (<, ≥grade-2). The main safety analysis included toxicities which had occurred in at least five males and females in each trial arm to allow interaction analyses:

leucopenia/neutropenia, infection, renal toxicity. For each type of toxicity, we estimated the treatment-by-sex interaction term using a logistic regression model stratified by trial and including age category, sex, treatment (main fixed effects) and treatment-by-sex interaction. We assessed the heterogeneity of the interaction across trials using a 3-order interaction term between treatment, sex and trial.

All estimates are given with 95% confidence intervals (95%CI) and two-sided p-values. Data collection and statistical analyses were performed using SAS Software 9.3. *Coxme* and *Meta* R packages for R version 3.0.2 (http://www.R-project.org) were used respectively to perform Cox regression models with random treatment effects and forest plots. The results are reported according to PRISMA-IPD recommendations.[24]

RESULTS

Trials description

In addition to the EE99-R1 trial[1], we identified three trials (EICESS92[25], IRS-IV[26] and an EORTC randomized phase-II trial in soft tissue sarcomas[27]) among 380 references of published papers and 37 studies registered on ClinicalTrials.gov (Figure-1). The EORTC trial was excluded because the individual patient data (survival and toxicity) were not available. We also excluded three randomized trials conducted exclusively in women (breast cancer[28], ovarian epithelial cancer[29] and endometrial adenocarcinoma[30]). Regarding the IRS-IV trial which compared three parallel groups, we considered the VAI and VAC arms, and excluded the third arm (vincristine-ifosfamide-etoposide arm). Actualization of the literature search in November 2016 did not identify any other trial fulfilling the inclusion criteria.

The three RCTs retained were high-quality phase III trials (See Supplemental Methods S1) comparing cyclophosphamide to ifosfamide in multi-drug combinations administered as first-line treatment (Table-1). Sex was considered as a stratification variable in these three trials.

The dose ratio of ifosfamide/cyclophosphamide ranged from 4 to 5. In total, 1528 patients were included, 773 in the cyclophosphamide arm and 755 in the ifosfamide arm. The EE99-R1 trial represented 56% of the total number of patients. These trials were all conducted in sarcomas (Ewing sarcoma, rhabdomyosarcoma and undifferentiated sarcomas). They included children, adolescents and young adults, aged <15 years in 66% of the patients (Table-2).

Survival analysis

With a median follow-up of 6.8 years [Q1-Q3, 4.5-8.9] (5.9 and 8.0 years in EE99-R1 and the validation set containing EICESS92 and IRS-IV, respectively), we observed 424 disease failures (i.e. PFS events: 224 and 200 in EE99-R1 and the validation set, respectively; progression or relapse in 395 patients and death as first event in 29, including 6 treatment-related deaths, 9 from disease progression, 9 other causes and 5 unknown causes). There were 325 deaths overall (171 and 154 in EE99-R1 and the validation set, respectively). The estimated treatment-by-sex interaction on PFS in EE99-R1 (HR=1.73, 95%CI 1.00-3.00, p-value=0.051) was not replicated in the validation set (n=672) using the one-stage model (EICESS92+IRS-IV, HR=0.97, 95%CI 0.55-1.72, p=0.93, Figure-2), with no heterogeneity between both trials (p=0.62). Interaction estimates were very similar in the sensitivity analyses (Table-3). In the same way, the estimated treatment-by-sex interaction in EE99-R1 for OS (HR=1.85, 95%CI 0.98-3.48, p=0.056) was not replicated in the validation set (HR=1.00, 95%CI 0.52-1.92, p=0.99, Supplemental Figure-1).

When the three RCTs were pooled, the estimated 5-year absolute PFS benefit associated with ifosfamide compared to cyclophosphamide was greater among males +6.0% (73.7% vs 67.9%), than females (+0.2%, 75.2% vs 75.0%, Figure-3). However, the overall estimate of treatment-by-sex interaction was not statistically significant (HR=1.31, 95%CI 0.89-1.95, p=0.17). Although a significant treatment-by-sex interaction was observed in EE99-R1

(p=0.051), this interaction was not statistically different to interaction terms estimated in EICESS92 and IRS-IV trials (p=0.36, Figure-2). This interaction estimate did not vary across age categories (p=0.88, Supplemental Figure S2). The sensitivity analyses yielded similar results (last column, Table-3). For OS (Supplemental Figure S3), the pooled estimate of the treatment-by-sex interaction was not statistically significant (HR=1.37, 95%CI 0.87-2.15, p=0.17). We observed neither heterogeneity across trials (p=0.35, Figure-4) nor across age categories (p=0.64, Supplemental Figure S4). Stable results were observed in the sensitivity analyses (Table-3).

Toxicity analysis

The frequencies of severe acute toxicities by sex and treatment arm are shown in Supplemental Table S1. At least one episode of severe acute neutropenia, infection and renal toxicity had occurred in 69.8%, 52.8% and 7.8% of patients, respectively. As illustrated in Supplemental Figures S5-7, no significant interaction was identified between sex and alkylating agent for leucopenia/neutropenia (OR=0.82, 95%CI 0.49-1.36, p=0.43), infection (OR=1.11, 95%CI 0.71-1.71, p=0.65), or renal toxicity (OR=1.71, 95%CI 0.76-3.85, p=0.19). These estimates did not significantly vary across trials (heterogeneity tests for leucopenia/neutropenia: p=0.81, infection: p=0.12, and renal toxicity: p=0.19). The main effects were reported because no interaction was found between treatment and sex. Compared to ifosfamide, patients receiving cyclophosphamide experienced more severe leucopenia/neutropenia ($OR_{cyclo\ vs\ ifo}$=1.47, 95%CI 1.14-1.88, p=0.003) and infections ($OR_{cyclo\ vs\ ifo}$=1.55, 95%CI 1.25-1.93, p<0.0001), but less renal toxicity ($OR_{cyclo\ vs\ ifo}$=0.71, 95%CI 0.48-1.06, p=0.098). Regardless of treatment arm, females developed significantly more severe leucopenia/neutropenia ($OR_{female\ vs\ male}$=1.39, 95%CI 1.08-1.79, p=0.013) and

infections ($OR_{female\ vs\ male}$=1.25, 95%CI 1.01-1.56, p=0.041) than males, but not significantly more severe renal toxicity ($OR_{female\ vs\ male}$=1.22, 95%CI 0.83-1.82, p=0.32).

DISCUSSION

Using an independent validation set of two RCTs (EICESS92 and IRS-IV), we did not replicate the treatment-by-sex interactions observed in the EE99-R1 trial on PFS and OS. No significant interactions were observed when the three trials were pooled, with no significant heterogeneity across age and trials. Similarly, we did not identify any treatment-by-sex interaction on leucopenia/neutropenia, infection and renal toxicity. Cyclophosphamide was significantly more hemato-toxic (leucopenia/neutropenia and infections) than ifosfamide. We also observed more hemato-toxicity in women than in males regardless of treatment arm.

This individual patient data meta-analysis is the first to assess a potential interaction between the type of alkylating agent and sex. Based on high-quality RCTs comparing cyclophosphamide to ifosfamide in both sex, with a total number of patients exceeding 1, 500 and long follow-up, it provides an unbiased estimate of the treatment-by-sex interaction. Finally, even though the search was not restricted to age or to a specific type of cancer, these three trials included mainly pediatric and young adult patients, with Ewing sarcoma or rhabdomyosarcoma under first-line treatment. This probably reduces sources of heterogeneity across trials (e.g. pharmacodynamic differences, co-morbidity, etc.).

The EORTC trial [27] which randomized cyclophosphamide and ifosfamide as a single drug in advanced or metastatic soft-tissue sarcomas (n=135 patients) was not included in the MAIAGE study due to the lack of availability of individual survival or toxicity data after contacting the principal investigator. This study reported lower response rates in the cyclophosphamide arm than in the ifosfamide arm, especially in males (observed response rate of 0% and 11% in males treated with cyclophosphamide and ifosfamide, respectively,

and of 17% and 23% in females). Based on these data, we did not observe any significant heterogeneity of the treatment effect between sex (interaction test: p=0.12). In the three other randomized trials excluded (because they were based on women only, see Appendix) [28-30], a better prognosis was reported in two, in subgroups of women treated with ifosfamide [29,30] whereas the difference was not significant in the third trial.[28]

Our study had some limitations. First, none of the trials analyzed were initially designed to study a treatment-by-sex interaction. Due to the observed number of events in each trial and when pooled, the analyses could be underpowered to test the interaction with a standard statistical level ($p<0.05$), let alone to detect heterogeneity of the treatment-by-sex interaction across trials (e.g. infection analysis with marginal heterogeneity across trials, p=0.12). Although we did not validate a treatment-by-sex interaction on efficacy outcomes, our results do not conclusively rule out the existence of an interaction.

Second, in addition to the index trial, we identified only two other RCTs, which together contributed less than 50% of the total number of patients. We did not identify any other study comparing cyclophosphamide and ifosfamide, hence there is a paucity of independent trials. Finally, differences in population characteristics and in drug combinations in the backbone chemotherapy could impact the consistency of the estimates of treatment-by-sex interaction. Indeed, (i) rhabdomyosarcoma patients in IRS-IV were younger than Ewing sarcoma patients from the other two trials, and (ii) all IRS-IV patients received four additional courses with cyclophosphamide after the first eight courses allocated by randomization; in contrast, all patients also received ifosfamide as induction chemotherapy before randomization in both Ewing sarcoma trials.

Our findings concerning acute toxicity are consistent with previous reports in sarcoma and lymphoma patients treated with alkylating agents.[10–14] Differences in cytochrome P450-mediated drug metabolism between sex could explain these results. Cyclophosphamide and

ifosfamide are oxazaphosphorine alkylating prodrugs that are metabolized via different P450-catalyzed pathways: (i) 4-hydroxylation produces active alkylating agents and urotoxic acrolein via CYP2B6 for cyclophosphamide and CYP3A4 and CYP3A5 for ifosfamide, and (ii) N-dechloroethylation generates inactive metabolites and nephro- and neuro-toxic chloroacetaldehyde via CYP3A4 for cyclophosphamide and, to a much greater extent, CYP3A4 and CYP2B6 for ifosfamide.[31–33] Greater activity of CYP3A4 and CYP2B6 has been reported in females resulting in higher concentrations of toxic chloroacetaldehyde after ifosfamide infusion and consequently in a possible higher risk of severe neurotoxicity in females.[34–36] However, no cytochrome P450-related difference in hematologic toxicity between sex has previously been reported.

In conclusion, our meta-analysis did not show that the treatment effect of cyclophosphamide versus ifosfamide is influenced by sex, for either efficacy or toxicity. Therefore, recommending the choice of alkylating agent should not need be based on sex in children and young adults treated for sarcoma. Additional studies would be useful for long-term follow-up including fertility outcomes.

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FIGURE LEGENDS

Figure 1: Flow chart of trial selection process.

C=Cyclophosphamide, I=Ifosfamide, STS=Soft tissue sarcoma.

*The search strategy used the following search terms: "Ifosfamide"[Mesh] AND "Cyclophosphamide"[Mesh] AND ("Randomized Controlled Trial" [Publication Type] OR "Controlled Clinical Trial" [Publication Type]) in PubMed, "Ifosfamide" AND "Cyclophosphamide" in the Cochrane Library, and "Ifosfamide" AND "Cyclophosphamide" AND "Randomized" in the NIH clinical trials register (http://www.clinicaltrials.gov).

Notes: Euro-EWING99-R1 trial was not yet published when we conducted the systematic review, that is why it does not appear in the initial systematic review box. Actualization of the literature search in November 2016 did not identify any other trial fulfilling the inclusion criteria.

Figure 2: Forest plot of the hazard ratios (HR) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex using fixed effects model.

The hazard ratios (HRs) given on the right side represent the HR of the treatment-by-sex interaction (HRCyclo/Ifo in males/ HRCyclo/Ifo in females) estimated independently for each trial, in the validation set and in the pooled dataset, by the one-stage model, stratified by trial and sex, and including treatment (cyclophosphamide vs. ifosfamide) and age (< 12, 12-18, and >18 years) as the main fixed effects. The heterogeneity of the interaction across trials was assessed using a 3-order interaction term. The center of each square represents the HR for individual trials and for the validation set (EICESS92 + IRS-IV) and the corresponding

horizontal line its 95% confidence interval (CI). The area of squares is proportional to the amount of information obtained from the trial. The center of the black diamond represents the overall HR and the extremities of the diamond represent its 95% CI, both estimated from the pooled dataset.

Figure 3: Stratified progression-free survival (PFS) curves according to sex and alkylating agent (cyclophosphamide or ifosfamide) when the 3 RCTs were pooled (n=1528).

The 5-year absolute PFS benefit associated with ifosfamide (Ifo) compared to cyclophosphamide (Cyclo) was estimated at 6% in males (73.7% vs. 67.9%), whereas females receiving ifosfamide or cyclophosphamide had similar PFS (75.2% vs. 75.0%, difference=0.2%).

SUPPLEMENTAL MATERIAL LEGENDS

1. Supplementary methods

Supplemental Methods S1: Procedure of data checking

Supplemental Methods S2: Statistical methods for sensitivity analyses

2. Supplementary results of survival analyses

Supplemental Figure S1: Forest plot of the hazard ratios (HR) of death (overall survival) in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex using fixed effects models.

Supplemental Figure S2: Forest plot of the hazard ratios (HR) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3 trials were pooled.

Supplemental Figure S3: Stratified overall survival (OS) curves according to sex and alkylating agent (cyclophosphamide or ifosfamide) when the 3 trials were pooled.

Supplemental Figure S4: Forest plot of the hazard ratios (HR) of overall survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3 trials were pooled.

3. Detailed results of toxicity analyses

Supplemental Table S1: Number of patients in each trial who experienced at least one episode of severe acute toxicity by sex and by treatment arm.

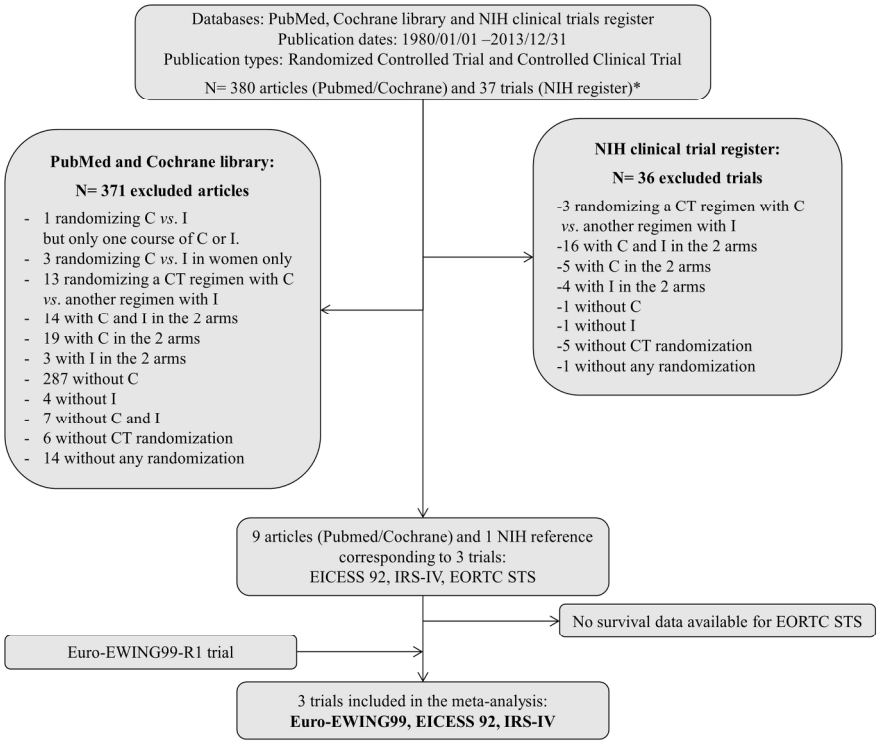
Supplemental Figure S5: Forest plot of the odd ratios (OR) of leucopenia/neutropenia in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

Supplemental Figure S6: Forest plot of the odd ratios (OR) of infection in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

Supplemental Figure S7: Forest plot of the odd ratios (OR) of renal toxicity in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

4. Description of the randomized controlled trials comparing alkylating agents, not included in the meta-analysis

Supplemental Table S2: Information extracted from the 3 randomized trials conducted in women and not included in the meta-analysis



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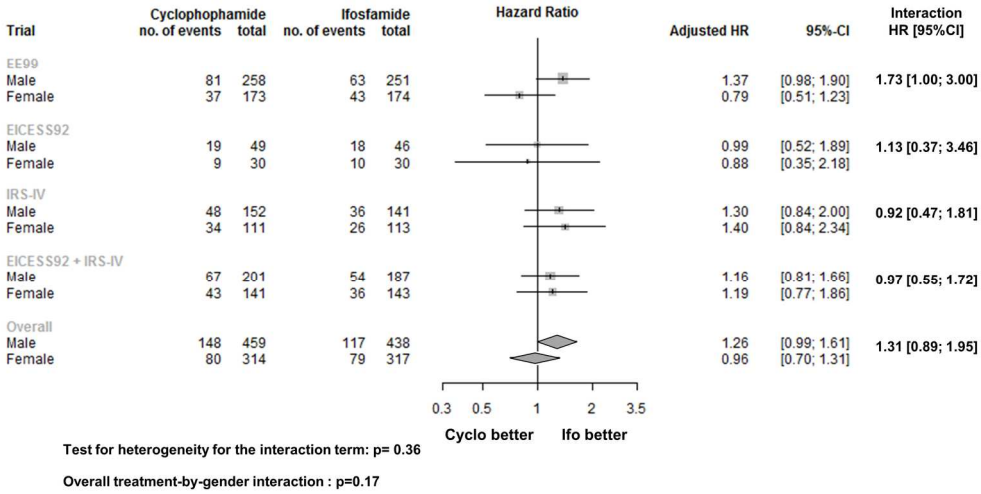

Figure 2: Forest plot of the hazard ratios (HR) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by gender using fixed effects model.

The hazard ratios (HRs) given on the right side represent the HR of the treatment-by-gender interaction (HRCyclo/Ifo in males/ HRCyclo/Ifo in females) estimated independently for each trial, in the validation set and in the pooled dataset, by the one-stage model, stratified by trial and gender, and including treatment (cyclophosphamide vs. ifosfamide) and age (< 12, 12-18, and >18 years) as the main fixed effects. The heterogeneity of the interaction across trials was assessed using a 3-order interaction term. The center of each square represents the HR for individual trials and for the validation set (EICESS92 + IRS-IV) and the corresponding horizontal line its 95% confidence interval (CI). The area of squares is proportional to the amount of information obtained from the trial. The center of the black diamond represents the overall HR and the extremities of the diamond represent its 95% CI, both estimated from the pooled dataset.

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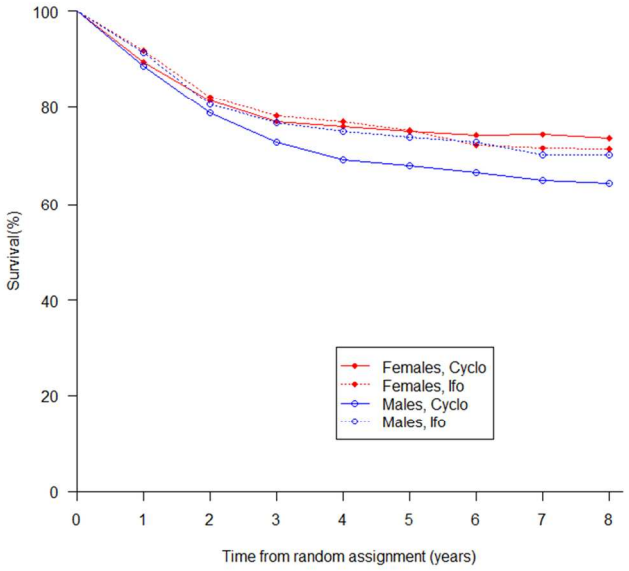

No. of deaths / person-years by period

Females-Ifo	25/295	29/260	11/223	3/192	4/172	6/138	1/110	0/87
Females-Cyclo	33/294	24/258	13/233	3/203	2/182	2/161	0/136	1/107
Males-Ifo	37/416	45/359	15/318	7/284	4/243	3/214	6/171	0/125
Males-Cyclo	51/424	43/360	25/311	14/269	4/238	4/201	4/160	1/119

Figure 3: Stratified progression-free survival (PFS) curves according to gender and alkylating agent (cyclophosphamide or ifosfamide) when the 3 RCTs were pooled (n=1528).

The 5-year absolute PFS benefit associated with ifosfamide (Ifo) compared to cyclophosphamide (Cyclo) was estimated at 6% in males (73.7% vs. 67.9%), whereas females receiving ifosfamide or cyclophosphamide had similar PFS (75.2% vs. 75.0%, difference=0.2%).

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TABLE 1 Characteristics of selected randomized clinical trials with regimens comparing cyclophosphamide versus ifosfamide.

Trial[ref]	Accrual period	Type of trial and design	N	Median follow-up [Q1-Q3]	Inclusion criteria			Eligibility criteria for randomization	Randomized regimens		Primary endpoint	Results of ITT† analysis
					Pathology‡	Primary tumor site	Age (years)		Ifo (dose/3w)	Cyclo (dose/3w)		
EE99-R1[1]	2000-2010	Multicentric Phase III Non-inferiority	856	5.9 [3.8; 8.0]	EWS	Bone or soft tissue	< 50	Localized tumors With a good response to preoperative CT*	7 VAI (3 g/m²x2)	7 VAC (1.5 g/m²x1)	3y-EFS	78% (VAI) 75% (VAC)
EICESS92[25]	1992-1999	Multicentric Phase III Non-inferiority	155	8.3 [6.9; 10.6]	ESFT	Bone	< 35	Localized tumors of less than 100mL	10 VAIA (2 g/m²x3)	10 VACA (1.2 g/m²x1)	3y-EFS	74% (VAIA) 73% (VACA)
IRS-IV[26]	1991-1997	Multicentric Phase III Superiority	517	8.0 [5.5; 9.9]	RMS, undifferentiated sarcoma	Soft tissue	< 21	Localized tumors**	8 VAI ◊ (1.8 g/m²x5)	8 VAC (2.2 g/m²x1)	3y-EFS	77% (VAI) 73% (VAC)

N: number of randomized patients, Cyclo: cyclophosphamide, Ifo: Ifosfamide, CT : chemotherapy, VAI : vincristine, dactinomycin, ifosfamide, VAC: vincristine, dactinomycin, cyclophosphamide, VAIA: vincristine, dactinomycin, ifosfamide, adriamycin, VACA: vincristine, dactinomycin, cyclophosphamide, adriamycin, EFS: event-free survival, Q1: first quartile, Q3: third quartile.
‡: EWS: Ewing sarcoma, ESFT: Ewing sarcoma family of tumors, RMS: rhabdomyosarcoma.
†: Intention to Treat. , w: week, y: year
* patients with either a good histologic response to preoperative treatment (<10% cells), or a small tumor (< 200 mL) resected at diagnosis or with radiotherapy alone as local treatment.
** after exclusion of patients with completely resected paratesticular tumors, completely resected or microscopic residual disease of orbit or eyelid tumors, pre-existing renal abnormalities.

TABLE 2 Characteristics of randomized patients in each trial included in the meta-analysis.

	EE99-R1		EICESS92		IRS-IV		Pooled dataset	
	VAI (n=425)	VAC (n=431)	VAIA (n=76)	VACA (n=79)	VAI (n=254)	VAC (n=263)	Ifo arm (n=755)	Cyclo arm (n=773)
Sex								
- male	251	258	46	49	141	152	438	459
- female	174	173	30	30	113	111	317	314
Age (years)								
Median	14.0	14.6	15.4	13.8	6.0	5.0	11.8	12.0
[0 ; 10[	120	99	17	18	172	190	309	307
[10 ; 15[	127	127	19	31	54	39	200	197
[15 ; 20[	88	107	23	17	28	32	139	156
≥20	90	98	17	13		2	107	113
Pathology								
- ESFT	415	416	73	77			488	493
- RMS					234	248	234	248
- Other bone sarcoma	1	1	1				2	1
- Other STS	10	14	2	2	20	15	32	31
Tumor stage								
- Localized disease	425	430	72	78	244	253	741	761
- Metastatic disease		1	3	1			3	2
- NA			1		10	10	11	10
Number of events	106	118	28	28	62	82	196	228
- Progression/relapse	*102*	*115*	*27*	*27*	*55*	*69*	*184*	*211*
- Death as first event	*4*	*3*	*1*	*1*	*7*	*13*	*12*	*17*
Number of deaths	83	88	18	21	51	64	152	173

VAI: vincristine, dactinomycin, ifosfamide, VAC: vincristine, dactinomycin, cyclophosphamide, VAIA: vincristine, dactinomycin, ifosfamide, adriamycin, VACA: vincristine, dactinomycin, cyclophosphamide, adriamycin, Ifo: ifosfamide, Cyclo: cyclophosphamide, CT: chemotherapy, ESFT: Ewing sarcoma family of tumors, RMS: rhabdomyosarcoma, STS: soft tissue sarcoma, NA: not applicable.

TABLE 3 Estimate of the hazard ratio of the treatment-by-gender interaction term for progression-free survival and overall survival for EE99-R1 (training set), EICESS92 + IRS-IV (validation set) and the pooled dataset in the main and sensitivity analyses.

	Training set EE99-R1 (n=856)	Validation set EICESS92 + IRS-IV (n=672)	Pooled analysis EE99-R1 + EICESS92 + IRS-IV (n=1528)
	HR (95%CI)	HR (95%CI)	HR (95%CI)
Progression-free survival			
- Main analysis: OSM, fixed effects, age category	1.73 (1.00-3.00), p=0.051	0.97 (0.55-1.72), p=0.93	1.31 (0.89;1.95), p=0.17
- Sensitivity analyses			
* OSM, random effects, age category	1.73 (1.00-3.00), p=0.051	0.98 (0.55-1.73), p=0.93	1.32 (0.89;1.95), p=0.17
* OSM, fixed effects, age continuous	1.71 (0.98-2.96), p=0.057	0.96 (0.55-1.71), p=0.90	1.31 (0.89-1.95), p=0.17
* PWT, fixed effects, age category		0.97 (0.55-1.73), p=0.92	1.32 (0.88;1.96), p=0.18
Overall survival			
- Main analysis: OSM, fixed effects, age category	1.85 (0.98-3.48), p=0.056	1.00 (0.52-1.92), p=0.99	1.37 (0.87;2.15), p=0.17
- Sensitivity analyses			
* OSM, random effects, age category	1.85 (0.98-3.48), p=0.056	1.00 (0.52-1.93), p=1.00	1.37 (0.87;2.16), p=0.17
* OSM, fixed effects, age continuous	1.80 (0.96-3.38), p=0.068	0.99 (0.51-1.91), p=0.98	1.37 (0.87;2.16), p=0.17
* PWT, fixed effects, age category		0.99 (0.51-1.91), p=0.98	1.37 (0.87;2.16), p=0.17

HR: hazard ratio of the treatment-by-gender interaction term (HR Cyclo vs. Ifo in males / HR Cyclo vs. Ifo in Females)
95%CI: 95% Confidence Interval
OSM: one-stage model; PWT: pooling of within-trial covariate interactions model; age category: <12 years, [12-18] years and >18 years

Supplementary material

1. **Supplementary methods**

   Supplemental Methods S1: Procedure of data checking

   Supplemental Methods S2: Statistical methods for sensitivity analyses

2. **Supplementary results of survival analyses**

3. **Detailed results of toxicity analyses**

4. **Description of the randomized controlled trials comparing alkylating agents, not included in the meta-analysis**

1. Supplementary methods

Supplemental Methods S1: Procedure of data checking

We have checked the data according to a standardized procedure[1]. Missing values and discrepancies were discussed with the trialists. Randomization validity was assessed by checking the patterns of treatment allocation and the balance in baseline characteristics between treatment groups. Definition of population set was evaluated for each trial to perform the meta-analysis according to the intention-to-treat principle. Patients follow-up was also compared between treatment groups. Each trial was then reanalyzed and the analyses were sent to the trialists for validation.

A. Randomization validity

Curves representing cumulative accrual were plotted and compared between treatment arms: no bias was observed. Among the selected trials, an imbalance between the baseline characteristics of the treatment arms was not detected (See Table 2).

B. Definition of the population sets

Respect of the intention-to-treat principle was requested for randomized trials even if some patients were excluded in the initial analyses of the trial. Overall, 65 randomized patients had been excluded in the initial trial publications, all in the IRS-IV trial. These 65 patients were included in the meta-analysis.

[1] Stewart LA, Clarke MJ on behalf of the Cochrane Working Group on meta-analyses using individual patient data. Practical methodology of meta-analyses (overviews) using updated individual patients data. Stat Med 1995;14:2057-2079.

C. Follow-up

For each treatment arm, reverse Kaplan-Meier curves were plotted: no bias was observed. Median follow-up was 6.8 years [Q1:4.5; Q3:8.9] in the pooled dataset and there was no difference between treatment arms within each trial (EE99-R1: 5.9 and 6.0 for VAC and VAI, respectively. EICESS92: 8.2 and 8.3 for VAIA and VACA, respectively. IRS-IV: 7.7 and 8.1 for VAI and VAC, respectively).

Supplemental Methods S2: Statistical methods for sensitivity analyses

Several pre-specified sensitivity analyses were performed:

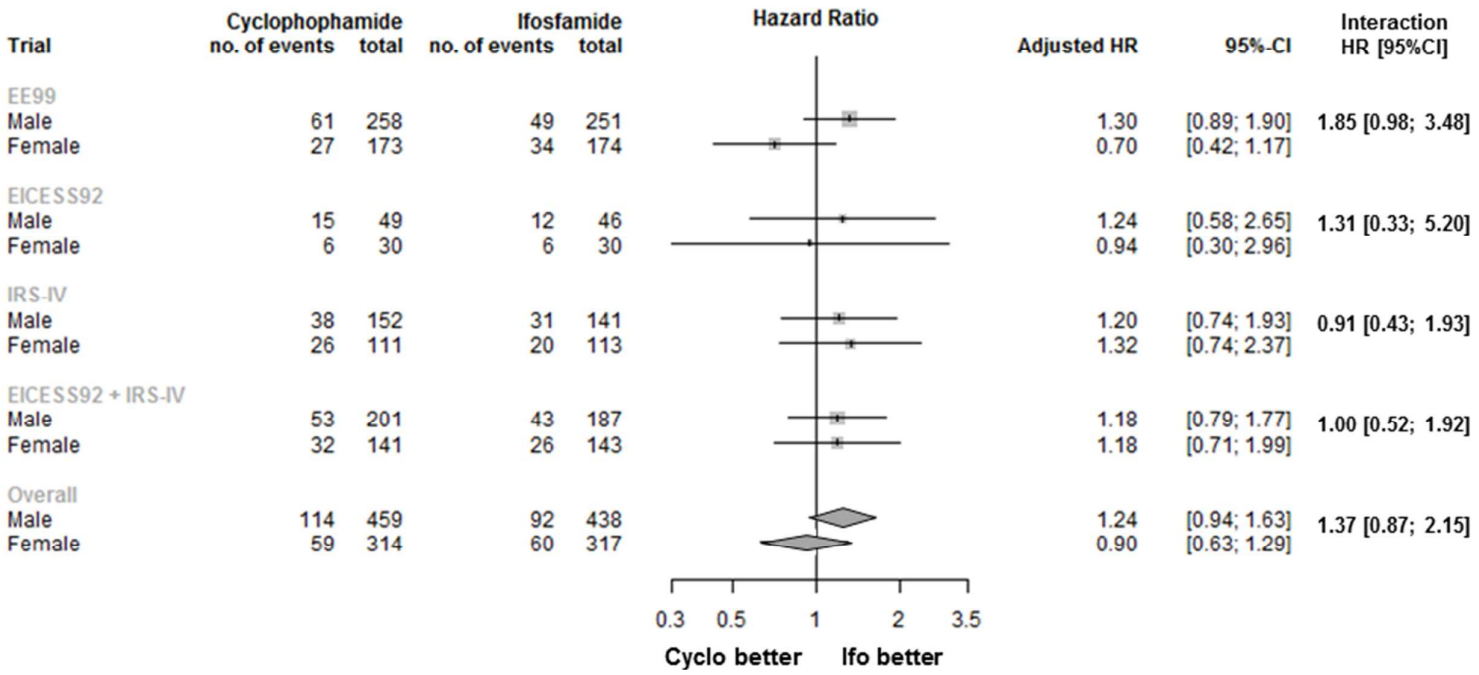
(i) The addition of a study-specific random component for the treatment effect in the one-stage method (OSM);

(ii) The impact of a misspecification of age was evaluated by including age as a continuous covariate in the OSM;

(iii) We used the "two-stage" approach to assess the overall treatment-by-sex interaction ("pooling within-trial covariate interactions" method, PWT).[20] We estimated interaction coefficients independently within each trial using multivariable Cox regression models, and then pooled them using the inverse-variance technique with fixed effects.[37]

1. Supplementary results of survival analyses

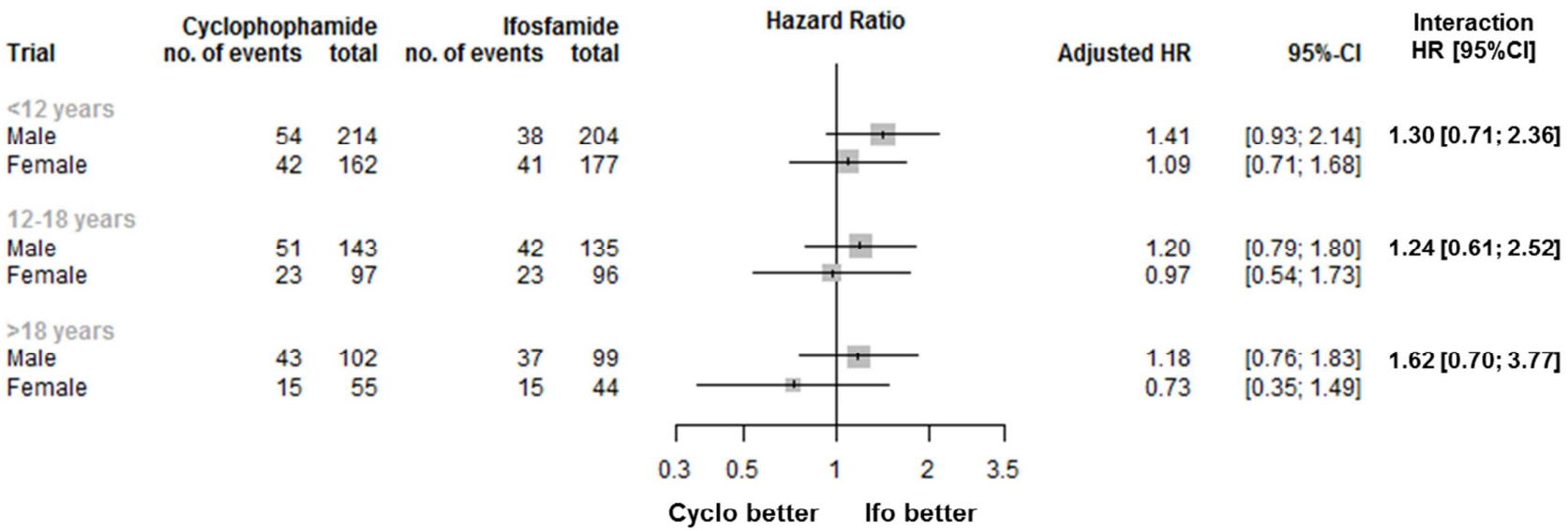
Supplemental Figure S1: Forest plot of the hazard ratios (HR) of death (overall survival) in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex using fixed effects models.



Test for heterogeneity for the interaction term: p= 0.35

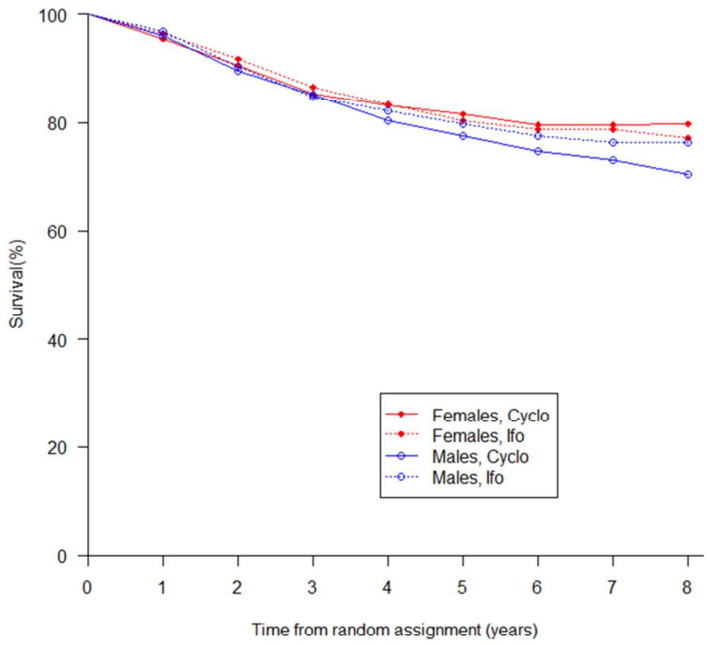
Overall treatment-by-gender interaction : p=0.17

Supplemental Figure S2: Forest plot of the hazard ratios (HR) of progression-free survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3 trials were pooled.



Test for heterogeneity for the interaction term: p= 0.88

Supplemental Figure S3: Stratified overall survival (OS) curves according to sex and alkylating agent (cyclophosphamide or ifosfamide) when the 3 trials were pooled.

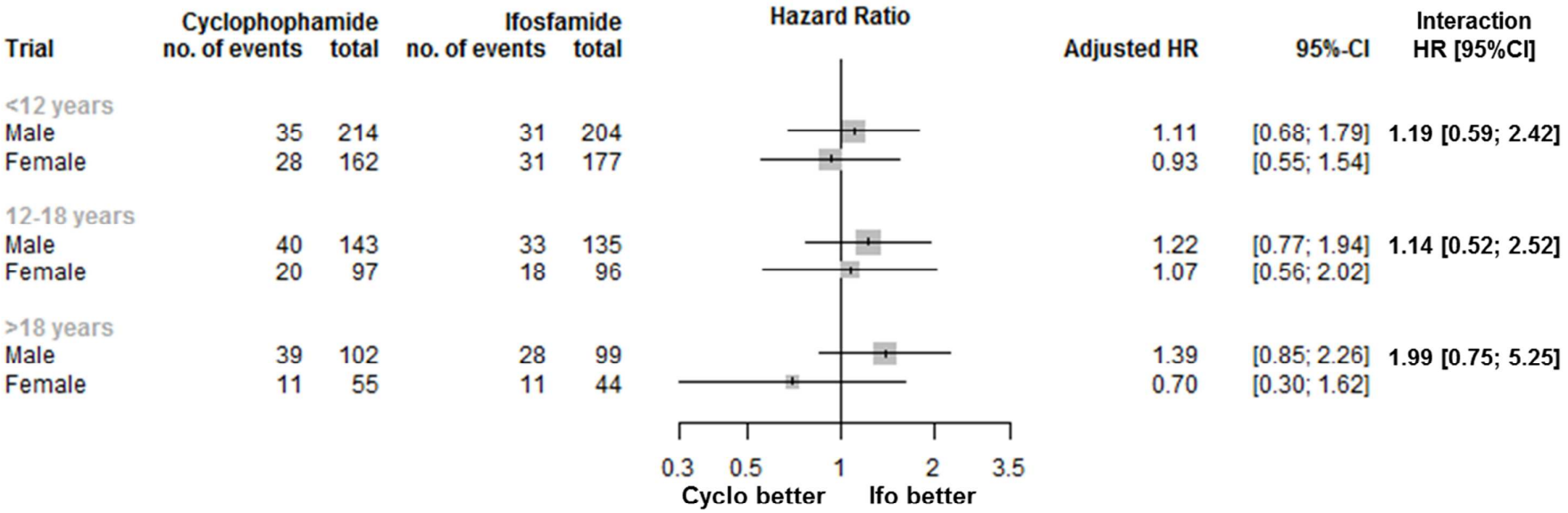


No. of deaths / person-years by period

Females-Ifo	11/303	14/281	14/248	8/211	7/184	3/150	0/123	2/97
Females-Cyclo	14/304	15/283	15/257	6/220	4/196	4/172	0/145	0/115
Males-Ifo	14/427	27/398	23/357	10/313	8/270	6/232	3/186	0/139
Males-Cyclo	18/439	28/402	18/358	18/314	10/273	8/230	4/184	5/137

The 5-year absolute OS benefit associated with ifosfamide (Ifo) compared to cyclophosphamide (Cyclo) was estimated at +2.2% in males (79.7% vs. 77.5%) and -1.1% in females (80.4% vs. 81.5%).

Supplemental Figure S4: Forest plot of the hazard ratios (HR) of overall survival in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex for each age category (<12 years, 12-18 years, >18 years) using fixed effects models when the 3 trials were pooled.



Test for heterogeneity for the interaction term: p= 0.64

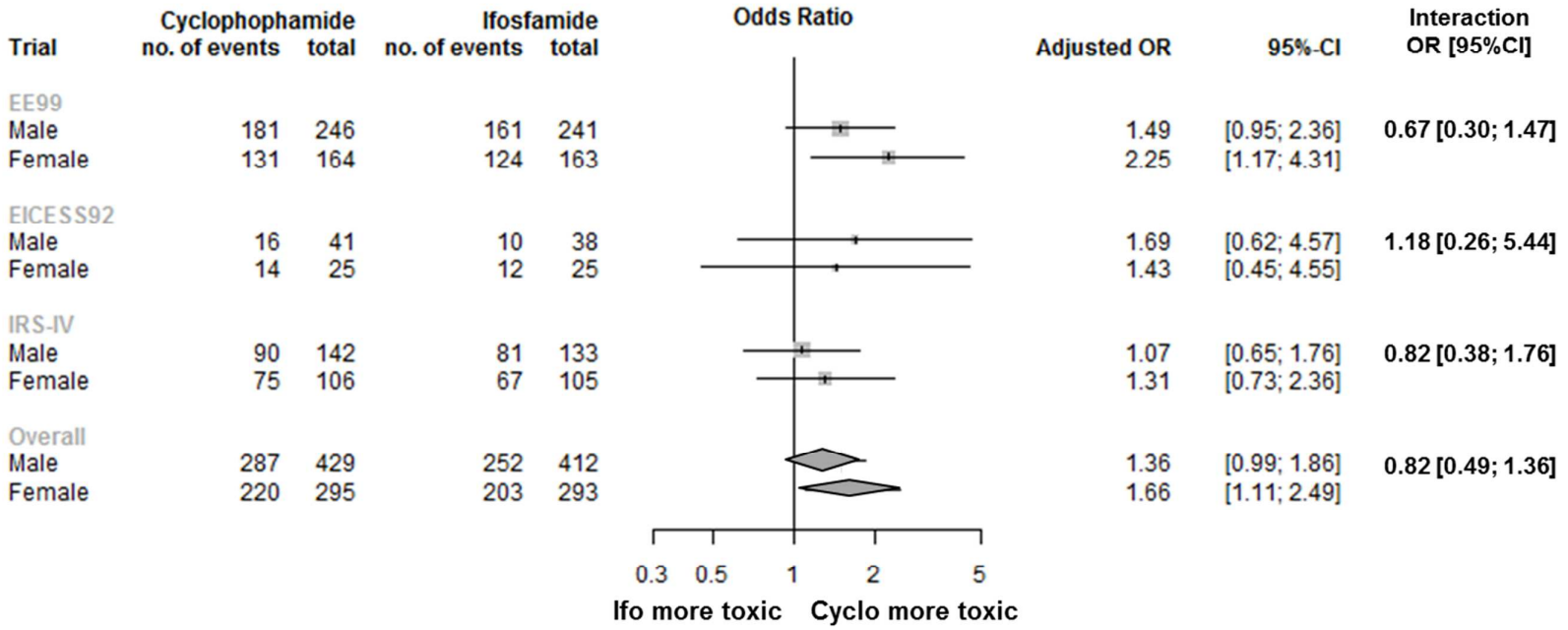
2. Detailed results of toxicity analyses

Supplemental Table S1: Number of patients in each trial who experienced at least one episode of severe acute toxicity by sex and by treatment arm.

Acute toxicity	Sex	Treatment	Number of patients with acute toxicity / number of patients with available information (%)		
			EE99-R1 (n=814)	EICESS92 (n=129)	IRS-IV (n=486)
Leucopenia/neutropenia	Female	VAC	131 / 152 (86.2)	14 / 25 (56.0)	75 / 106 (70.8)
		VAI	124 / 155 (80.0)	12 / 25 (48.0)	67 / 105 (63.8)
	Male	VAC	181 / 234 (77.4)	16 / 40 (40.0)	90 / 142 (63.4)
		VAI	161 / 225 (71.6)	10 / 37 (27.0)	81 / 133 (60.9)
Infection	Female	VAC	90 / 161 (55.9)	13 / 25 (52.0)	73 / 106 (68.9)
		VAI	89 / 161 (55.3)	9 / 25 (36.0)	56 / 105 (53.3)
	Male	VAC	127 / 246 (51.6)	20 / 40 (50.0)	87 / 142 (61.3)
		VAI	88 / 240 (36.7)	15 / 37 (40.5)	75 / 133 (56.4)
Renal toxicity*	Female	VAC	8 / 160 (5.0)	5 / 24 (20.8)	5 / 106 (4.7)
		VAI	22 / 160 (13.8)	6 / 25 (24.0)	5 / 105 (4.8)
	Male	VAC	13 / 246 (5.3)	9 / 40 (22.5)	7 / 142 (4.9)
		VAI	12 / 239 (5.0)	5 / 38 (13.2)	13 / 133 (9.8)
Thrombocytopenia	Female	VAC	79 / 161 (49.1)	4 / 25 (16.0)	57 / 106 (53.8)
		VAI	72 / 161 (44.7)	0 / 25 (0.0)	38 / 105 (36.2)
	Male	VAC	102 / 245 (41.6)	4 / 40 (10.0)	70 / 142 (49.3)
		VAI	64 / 241 (26.6)	1 / 37 (2.7)	36 / 133 (27.1)
Mucositis	Female	VAC	6 / 160 (3.8)	3 / 25 (12.0)	59 / 106 (55.7)
		VAI	6 / 160 (3.8)	0 / 24 (0.0)	40 / 105 (38.1)
	Male	VAC	5 / 246 (2.0)	3 / 39 (7.7)	50 / 142 (35.2)
		VAI	5 / 240 (2.1)	2 / 37 (5.4)	55 / 133 (41.4)
Diarrhea	Female	VAC	1 / 160 (0.6)	1 / 12 (8.3)	18 / 106 (17.0)
		VAI	5 / 160 (3.1)	0 / 14 (0.0)	9 / 105 (8.6)
	Male	VAC	4 / 246 (1.6)	1 / 23 (4.3)	18 / 142 (12.7)
		VAI	1 / 240 (0.4)	0 / 26 (0.0)	12 / 133 (9.0)
Liver toxicity	Female	VAC	7 / 160 (4.4)	1 / 25 (4.0)	15 / 106 (14.2)
		VAI	11 / 159 (6.9)	2 / 24 (8.3)	9 / 105 (8.6)
	Male	VAC	15 / 245 (6.1)	3 / 38 (7.9)	23 / 142 (16.2)
		VAI	9 / 239 (3.8)	0 / 37 (0.0)	8 / 133 (6.0)
Central neurologic toxicity	Female	VAC	1 / 160 (0.6)	2 / 24 (8.3)	5 / 106 (4.7)
		VAI	4 / 160 (2.5)	0 / 24 (0.0)	7 / 105 (6.7)
	Male	VAC	2 / 244 (0.8)	0 / 39 (0.0)	7 / 142 (4.9)
		VAI	3 / 240 (1.3)	0 / 36 (0.0)	6 / 133 (4.5)
Peripheral neurologic toxicity	Female	VAC	11 / 159 (6.9)	3 / 25 (12.0)	26 / 106 (24.5)
		VAI	15 / 159 (9.4)	1 / 24 (4.2)	25 / 105 (23.8)
	Male	VAC	17 / 245 (6.9)	3 / 39 (7.7)	35 / 142 (24.6)
		VAI	8 / 240 (3.3)	2 / 37 (5.4)	34 / 133 (25.6)
Cardiac toxicity	Female	VAC	3 / 133 (2.3)	5 / 23 (21.7)	2 / 106 (1.9)
		VAI	9 / 143 (6.3)	4 / 22 (18.2)	2 / 105 (1.9)
	Male	VAC	6 / 210 (2.9)	8 / 36 (22.2)	3 / 142 (2.1)
		VAI	6 / 208 (2.9)	8 / 33 (24.2)	1 / 133 (0.8)

VAI: vincristine, dactinomycin, ifosfamide, VAC: vincristine, dactinomycin, cyclophosphamide
Adverse events were evaluated using the NCI CTCAE-v2 scale in the EE99-R1 and EICESS92 trials, and NCI CTCAE-v1 scale in the IRS-IV trial.
*Severe renal toxicity (grade 2 or more): at least one episode of increased plasmatic creatinine > 1.5 baseline, or a glomerular filtration rate decrease <60ml/min/1.73m² or a tubular phosphate reabsorption decrease <80%.

Supplemental Figure S5: Forest plot of the odd ratios (OR) of leucopenia/neutropenia in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.

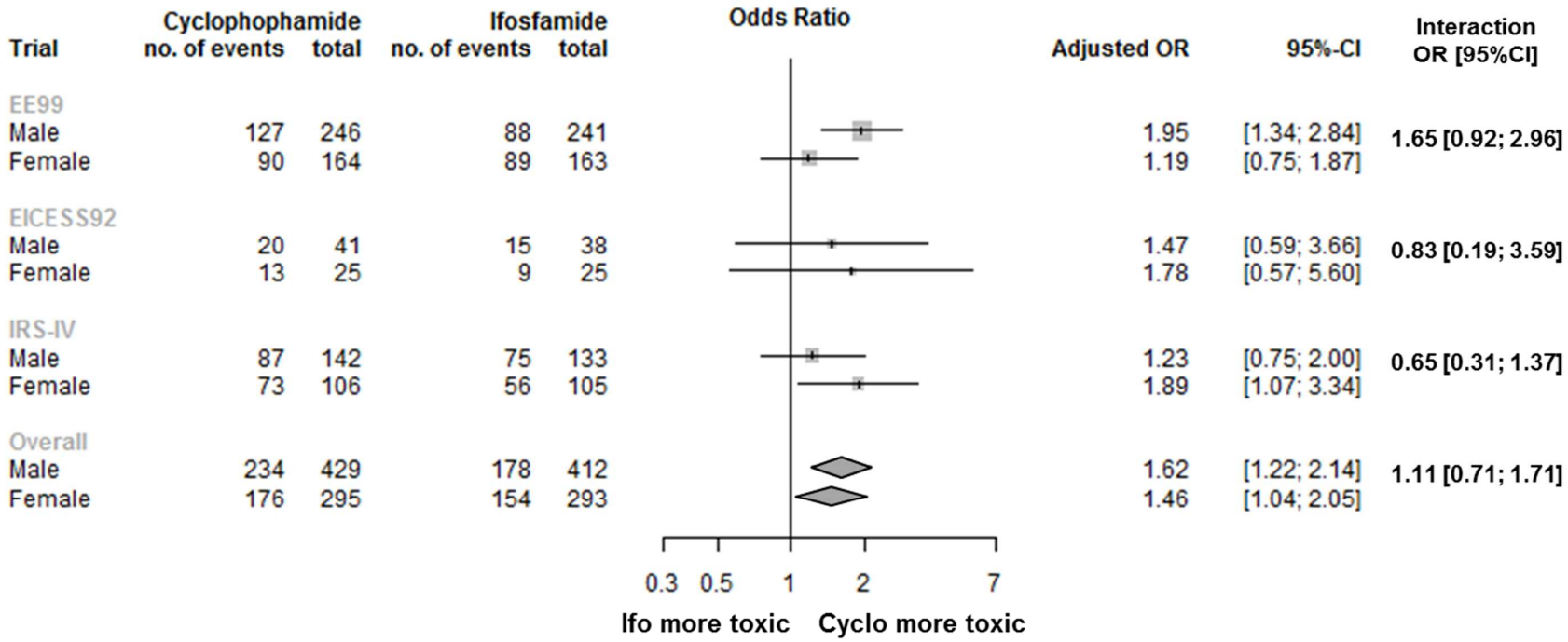


Test for heterogeneity for the interaction term: p= 0.81

Overall treatment-by-gender interaction : p=0.43

The Odd Ratios (ORs) given on the right side represent the OR of the treatment-by-sex interaction ($OR_{Cyclo/Ifo\ in\ males}$/ $OR_{Cyclo/Ifo\ in\ females}$) estimated independently for each trial and in the pooled dataset, using the logistic regression model, stratified by trial and sex, and including treatment (cyclophosphamide *vs*. ifosfamide) and age (< 12, 12-18, and >18 years) as the main fixed effects. Heterogeneity of the interaction (treatment x sex) across trials was assessed using the 3-order interaction term.

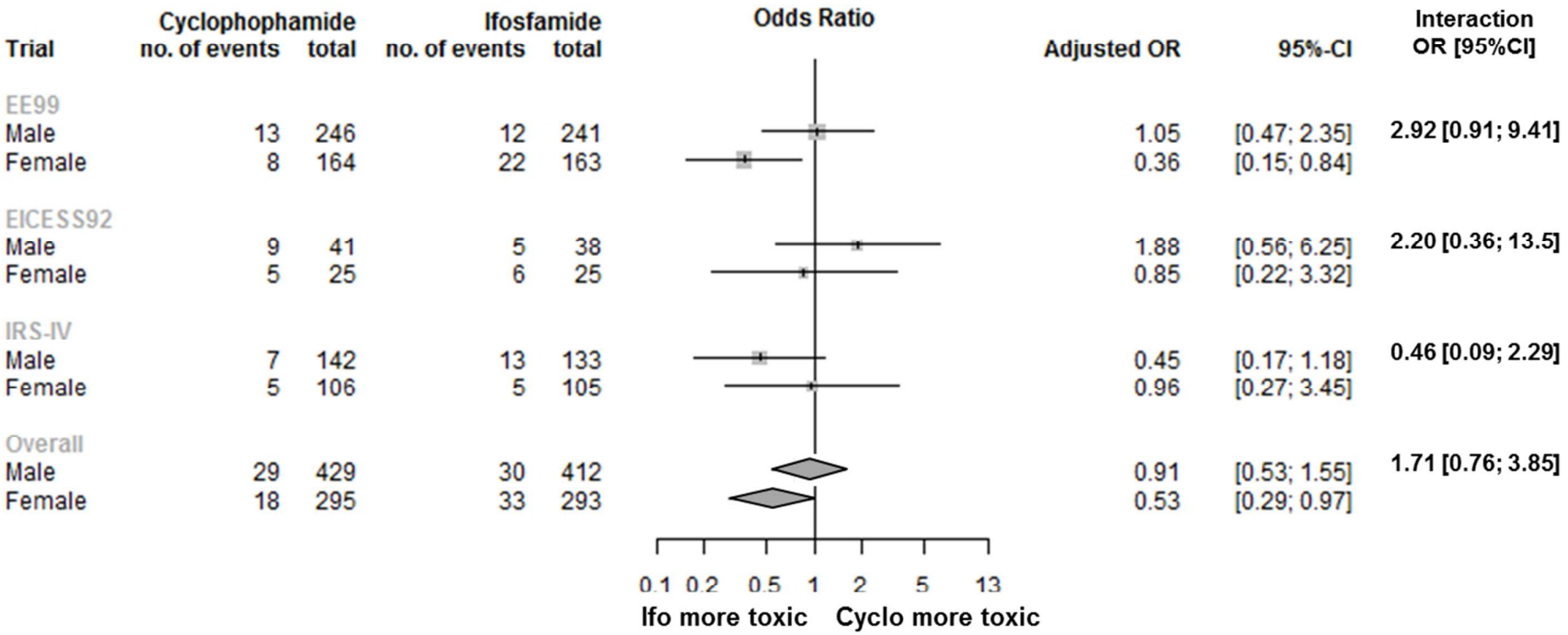
Supplemental Figure S6: Forest plot of the odd ratios (OR) of infection in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.



Test for heterogeneity for the interaction term: p= 0.12

Overall treatment-by-gender interaction : p=0.65

Supplemental Figure S7: Forest plot of the odd ratios (OR) of renal toxicity in the cyclophosphamide (Cyclo) arm versus the ifosfamide (Ifo) arm by sex when the 3 trials were pooled.



Test for heterogeneity for the interaction term: p= 0.19

Overall treatment-by-gender interaction : p=0.19

3. Description of the randomized controlled trials comparing alkylating agents, not included in the meta-analysis

Supplemental Table S2: Information extracted from the 3 randomized trials conducted in women and not included in the meta-analysis

Author	Pathology	Treatment arms	Number of patients	Response rate (CR or PR)	Progression-free survival (PFS)‡			Overall survival (OS)
Buzdar [28]	Breast carcinoma	FAC+BCG+levamisole	117	72.6%	Median time to progression: 17 months			Median OS: 21.4 months
		FAI+BCG+levamisole	49*	65.3%	Median time to progression: 17.8 months			Median OS: 23.5 months
Nishida [29]	Ovarian epithelial cancer	PAC	53	NA	3y-PFS: 84.9%	5y-PFS: 79.0%	10y-PFS: 67.8%	NA
		PAI	52	NA	3y-PFS: 88.5%	5y-PFS: 88.5%	10y-PFS: 81.1%	NA
Pawinski [30]	Adenocarcinoma of uterine corpus	Cyclo	29	6.9%	Median time to progression: 7 weeks			NA
		Ifo	32	12.5%	Median time to progression: 8 weeks			NA

FAC: 5-fluorouracil, adriamycin, cyclophosphamide, FAI: 5-fluorouracil, adriamycin, cyclophosphamide, PAC: cisplatin, epirubicin, cyclophosphamide, PAI: cisplatin, epirubicin, ifosfamide, Cyclo: cyclophosphamide, Ifo: ifosfamide, CR: complete response, PR: partial response, NA: not available

* The FAI arm was closed because of increased bladder toxicity observed with ifosfamide resulting in a greater number of patients in the FAC arm.

‡: no information on the precision of the estimate (standard error, confidence interval or number of at-risk patients) was reported.